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BRAIN PRESERVATION

WITH A RÉSUMÉ OF

SOME OLD AND NEW METHODS

BY

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By PIERRE A. FISH.

The brain, the organ of thought, complex in structure, the great co-ordinator of bodily functions, the master and yet the servant of the animal economy, has been the last of the viscera to receive careful preservation. The ancient Egyptians in their most *perfect* embalments "drew the brain through the nostrils partly with a piece of crooked iron and partly with the infusion of drugs." The other viscera upon removal were carefully cleansed and after proper treatment were replaced in the body, the brain apparently being the only part rejected.

The summary treatment of this important organ and the bad precedent thus established by the Egyptians retarded for a long time the development of any progressive ideas in this direction. From the time of the Egyptians down to near the close of the seventeenth century no advance but actual retrogression occurred in the art of preservation; this being due to some extent to the indifference of the nations in power at that time, but chiefly to the great religious opposition toward anything pertaining to science. During this dark period of scientific stagnation much has been lost that may never be recovered.

The crude and erroneous descriptions of the early anatomists justify the belief that their methods were but little superior to those that preceded, but the progress in those early years of embalming the body, marks also an advance, slight and inefficient perhaps, but nevertheless an advance, in the preservation of the brain itself; particularly so when the injection method came into use. To a Hollander, Frederic Ruysch, Professor of Anatomy, at Amsterdam from 1655 to 1717, belongs the honor of having originated and perfected this method to such an extent that his specimens are said to have been wonderfully life-like and to have aroused the admiration of the people of his age. The formula of his preservative was not divulged and the secret of its preparation died with him.

William Hunter did much to extend the practice of injection by producing some very beautiful specimens and the impetus thus given by these early anatomists has brought the method down to us with but few if any radical changes.

Admirable as these results were concerning the body as a whole, it became apparent that they were quite inadequate when a more thorough and accurate knowledge of brain morphology was demanded, thus it came about that greater care was used in the removal of the brain and special methods of treatment were devised, and the importance of technique became more and more emphasized, especially so within the last two or three decades.

The consistence of the brain coupled with the difficulty of its removal renders it a difficult organ to preserve. History gives good evidence that the advance in the knowledge of brain structure has been largely dependent upon improved methods of manipulation.

The purpose of hardening is to bring the brain into a proper condition for the continued study of either its fine or gross anatomy, the former usually requiring some special care in methods and after-treatment which may be dispensed with in the latter without apparent detriment.

For the study of the gross anatomy either wet or dry preparations may be available. The preference generally being given to the wet since they are more easily and quickly prepared and because they admit of further and careful dissection at any time after once being well hardened. A shrinkage in the tissues must necessarily occur during this process but it is not usually carried so far as in the case of the dry preparations. Nor is there such an unnatural color unless some colored preservative is employed. But there is the disadvantage of a possible ruination of the specimens by over-exposure to the air, evaporation or deterioration of the preservative and a consequent expense in renewing the same.

For the study of surface anatomy and of certain parts dissected out before the specimen is "dried," there is no reason why, if successfully prepared, the dry method would not answer most needs and have the further advantage of remaining permanent in the air.

Reil's method of preparing the brain : *

"Of the methods which I have employed in preparing brains those contained in the following directions answer best : (1). Let the brain be hardened in alcohol and then placed in a solution of carbonated or pure alkali, in the latter two days, in the former for a longer period, and then again hardened in alcohol if thus rendered too soft. The advantage of this method is that the fasciculi of nervous matter are more readily separable and the brown matter more distinguishable from the white than after simple maceration in alcohol ; the gray matter is rendered by the alkali of a blacker gray and assumes the consistence of jelly. (2). Let the brain be macerated in alcohol in which pure or carbonated potass. or ammonia, has been previously dissolved ; the contraction of the brain is lessened by this process. (3). Let the brain be macerated in alcohol from six to eight days and then its superficial dissection commenced, and the separation of the deeper parts continued, as the fluid in which the brain is kept immersed, penetrates its substance. This method appears to me better than the preceding, and would very likely be improved if the alcohol were rendered alkaline. The fibers in a brain thus prepared are more tenacious than otherwise, and the deeper parts are sooner exposed to the influence of the alcohol."

These methods are applicable chiefly for the macroscopic study of commissural relations and the general direction of fibers.

J. Müller in 1834 recommended the use of creosote water for the preservation of the brain and myel.

Alcohol is the oldest and most universal preservative employed. It has good "fixing" properties but needs considerable attention in order to produce the best results. For fixing, it is frequently used in conjunction with some of the various salts, or in case some non-alcoholic fixer is used, it supplements or completes the hardening thus begun. As a preservative it is generally used at the ordinary commercial strength —ninety to ninety-five per cent., although for most tissues eighty or even seventy-five per cent. seems to suffice.

On account of the continuous dehydration and the struct-

* Mayo's translation of Reil's Eighth Essay.

ural changes induced thereby, it is advisable to use not higher than ninety per cent. The great and unequal attractive power of alcohol for water, renders it necessary to begin with the lower grades. Otherwise the rapid withdrawal of the water before the alcohol can replace it, will cause shrinkage and the tearing or breaking down of the tissue. Immersion of a large specimen in a limited quantity of strong alcohol is likely to induce a rapid hardening of the surface, forming a crust through which the alcohol may cease to penetrate, causing a consequent maceration of the interior.

For general utility, economy and certainty of result, no reagent excels potassium bichromate in its action on nervous tissue. It is said that attention was called to this salt for hardening purposes by a Mr. Savory, some thirty or more years ago[16]. It is commonly used in a simple two or five per cent. solution or in the form of "Müllers" or "Erlicki's" liquids. The simple solution has of late come into greater prominence.

It is inexpensive ; it hardens slowly but thoroughly, with a minimum of distortion and leaves the specimen in a state of good consistency even if its action is prolonged. Its application is general ; it preserves the contours of large and irregular areas for the morphologist and maintains the proper relations of the structural elements for the histologist. A little chromic acid (one or two drops of a one per cent. solution) added to each thirty cubic centimeters of the bichromate will do no harm and will quicken the hardening. [13] All chromic salts impart a disagreeable and abnormal color to the specimens and for some purposes render them quite undesirable.

This[16] it is said may be obviated to some extent by hardening the tissue in the following mixture :

Potassium bichromate	6 grams
Potassium nitrate	4 grams
Water	180 c. c.

After-treatment with absolute alcohol is recommended by W. C. Krauss for decolorization. Unna advises peroxide of hydrogen. Lee mentions chloral hydrate in a one per cent.

solution, but this is declared by Gierke to be prejudical to the preservation of the tissues.

Corrosive sublimate is useful as a fixative either in an aqueous or alcoholic solution ; it is more soluble in the latter. Chaussier at the beginning of the nineteenth century recognized the antiseptic properties of this salt and since that time it has been quite extensively used as a preservative. Professor Robert Garner[8] with regard to his method says : " *We* let the brain fall from the skull into a hardening solution of bichloride of mercury, the strength about six ounces of the salt to the half gallon of water making a fluid of about 1.038 sp. gr. or the same as the brain itself, in which it consequently remains suspended in mid-fluid without pressure on any of its surfaces and becoming hard and solid without the contraction which takes place when spirit is used. "

Richardson[16] gives the following formula for the central nervous system :

Mercuric chlorid	2 grams
Alcohol (sp. gr. 83)	90 c. c.
Hydrochloric acid	2 c. c.

There are various inconveniences attending the use of this reagent, not the least of which are its corrosive action on anything metallic making it very necessary that all traces of it be washed out before any dissection is undertaken ; its caustic action on the hands is very marked ; precipitates often occur in the tissue and are a source of considerable annoyance to the histologist. Camphor renders the sublimate more soluble and if the tissue after its sublimate bath be brought into alcohol containing camphor the washing out of the salt is considerably expedited. Tincture of iodine is another agent useful in this respect. A little of it is added to the alcohol and as it dissolves out the sublimate, the color of the solution is weakened and the iodine is gradually renewed until the color no longer fades. The alcohol should be changed frequently. If the sublimate is not thoroughly removed from the tissues they become brittle.

The origin of the use of Zinc chlorid for neurological purposes is enveloped in considerable uncertainty. Bischoff* in a

*Die Grosshirnwindungen des Menschen. München. 1868. S. 11.

note says: "From a note in Gratiolet (Mémoire sur le plis cérébraux de l'homme. Paris. 1854, p. 11.) it is to be seen that a Parisian modeller, Stahl, likewise used the zinc chlorid for hardening brains, in order to make a cast of the same afterward, but it does not appear that Gratiolet employed the same process in his anatomical researches." Bischoff himself had used it for some years previous to 1868.

It is a deliquescent salt and specimens should not be left too long in its solution lest they soften. The hardening is continued in alcohol. Aqueous solutions are generally used since enough of the salt may be dissolved to support the brain. Broca[3] (1879), was perhaps the first to recommend it in an alcoholic solution (ten per cent.). It acts here as a very strong dehydrant, but its action is even if rapid, and with careful treatment no marked distortion results. It has also proven eminently satisfactory for histological work, but for this a five per cent. solution is apparently just as efficacious as the stronger. The specific gravity of a saturated alcoholic solution is not great enough to buoy the brain, and a bed of cotton is therefore necessary.

Glycerin makes a very efficient preservative. It is, however, generally utilized as an adjunct in methods more or less complex or for the immersion of specimens that have already been hardened.

Nitric acid in a ten or twelve per cent. solution has also been recommended; the specimen is to be immersed from twelve to fifteen days and turned frequently as the liquid is too dense to admit of its being entirely covered. This reagent is said to give the *toughest* of preparations.

Experiments were made in May, 1892, to determine approximately the relative loss of weight and girth of a number of sheep brains prepared in different ways. The girth was ascertained by measuring transversely around the brain at the level of the temporal lobes. This as well as the weight was determined at three stages during the course of hardening: first, when fresh; second, the intermediate stage, or before the specimen was brought into alcohol; third, after immersion in alcohol for a longer or shorter time. The accompanying table shows very concisely the results thus obtained.

Fluid.	Weight.			Girth.		
	Fresh.	*Intermediate.*	*Alcohol.*	*Fresh.*	*Intermediate.*	*Alcohol.*
1. Zinc chlorid, ... 200 grams, 70 per cent. Alcohol . 3000 c. c., Glycerin, ... 1200 c. c., Sp. gr. 1.05.	114 grams May 2, '92.	87 grams May 9, '92.	78 grams May 12.	16.4 cm.	15.2 cm.	14.5 cm.
2. 10 per cent. aqueous solution of Zinc chlorid, Sp. gr. 1.14,.	112 grams May 2.	93 grams May 24.	67 grams June 11.	16.8 cm.	15. cm.	14.5 cm.
3. Equal parts of a saturated aqueous sol. of Potassium bichromate and a 10 pr ct. aqueous sol. of Zinc chlorid.	110 grams May 2.	96 grams May 9.	71 grams June 11.	16.5 cm.	15.7 cm.	15.2 cm.
4. Saturated aqueous solution of Corrosive sublimate, Sp. gr. 1.05, . .	112 grams May 2.	118 grams May 24.	76 grams June 11.	16.9 cm.	16.9 cm.	14.9 cm.
5. Equal parts of saturated solutions of Potassium bichromate and Corrosive sublimate, (aqueous) . . .	100 grams May 2.	108 grams May 9.	75 grams June 11.	15.9 cm.	16.5 cm.	15.3 cm.
6. Saturated aqueous solution of Potassium bichromate, Sp. gr. 1.06,	101 grams May 2.	115 grams June 11.	94 grams Sept. 19.	15.9 cm.	16.6 cm.	16. cm.

The brain "fixed" in fluid No. 1 did not sink to the bottom of the vessel until after six days. Within ten days it had lost 36 grams in weight, and 1.9 centimeters in girth, and had become slightly distorted. The specimen in fluid No. 2 floated for more than a week; it also became somewhat distorted. The loss of weight was 45 grams, of girth 2.3 centimeters, being greater than in any of the others. Fluid No. 3 was very rapid in its action and produced a very firm preparation. The color was considerably lighter than in the ordinary bichromate specimens. The loss of weight was 39 grams, of of girth 1.3 centimeters. It should be noted with regard to fluid No. 4, that the weight increased 6 grams at the intermediate stage and that the girth was exactly the same as when fresh. At the third stage, however, there was a loss of 36 grams in weight, and of 2 centimeters in girth, due without doubt to the re-dissolving of the sublimate in the alcohol. Fluid No. 5 gave a better final test than did any of the preceding. There was an increase of 8 grams in weight and of 0.6 centimeter in girth at the intermediate stage. The loss of weight was 25 grams and of girth 0.6 centimeter. Treatment with fluid No. 6 left the brain nearest to its original weight and girth. There was a gain of 14 grams in weight and of 0.7 centimeter in girth at the intermediate stage. After more than four months from the date of its first treatment it had lost only 7 grams in weight and had *gained* 0.1 centimeter in girth. The bichromate is nearly insoluble in alcohol, and once having penetrated the tissue thoroughly, it remains; the replacement of the natural water of the tissues is so gradual that there is little or no chance for shrinkage, while the alcohol afterward helps to keep the salt in place if kept in the dark (Virchow). The alba and cinerea are quite markedly differentiated; and there always exists the abnormal but characteristic chromic color.

An ideal preservative would be one of about the same specific gravity as the brain itself, replacing gradually the natural fluids of the tissue with a simple fluid, or with a solution of some salt of equal density, and not markedly changing the natural color or size of the specimen.

There are two liquids which will cause the brain to retain approximately its normal size; one is glycerin which, after it has thoroughly infiltrated the hardened tissues, causes them to absorb moisture from the atmosphere and the natural fluid is thus artificially replaced by means of this hygroscopic agent. There must, however, be some limit to the preservative action, and the time may eventually come when enough water will have been absorbed to cause considerable deterioration. The other liquid is potassium bichromate which, as noted in the table, caused an actual "bloating" of the tissue, increasing both the weight and girth of the specimen, and imparting an undesirable as well as an unnatural color. The pia is a more or less inelastic and pervious membrane, and while on the one hand it may retard the penetration of the fluid, it serves a little later, in the case of the bichromate to restrain the "bloating" and keep the tissue within bounds. The pressure either from without or within, would tend to disturb the normal relations of the histological elements.

Brains from animals of the same species react differently although subjected to exactly the same course of treatment. The density of the tissue, the age and condition of the subject, the temperature and many other factors equally important, are causes which contribute to these varying results.

After considerable study and experimenting a fluid was devised, which, though not ideal in its effects, seems to answer the requirements of economy, fixation of the structural elements, differentiation of tissue, a minimum amount of distortion, firmness of texture, and rapidity of action.

The formula is as follows:

Water	400 c. c.
95% Alcohol	400 c. c.
Glycerin	250 c. c.
Zinc chlorid	20 grams.
Sodium chlorid	20 grams.

The specfic gravity of the mixture should be about 1.04, a little greater than that of the brain itself (1.038). The

slightly greater density of the fluid is believed to be more advantageous than otherwise, since it buoys the brain until the tissue has begun to harden and can partially support its own weight. The pressure is nearly enough equal on all sides to prevent any noticeable change of form. It is recommended that the cavities of the brain be filled with the mixture (cœlinjected) and if practicable the blood-vessels also injected. After an immersion of about three days the specimen should be transferred to equal parts of the foregoing mixture and seventy per cent. alcohol for a week or more, where on account of the lesser specific gravity it should rest upon a bed of absorbent cotton ; it is finally stored in 90% alcohol.

The addition of the zinc chlorid to the solution is to expedite the hardening, to differentiate the tissue, and to insure a more equable and penetrating action. Osler attributes the differential effect to the glycerin or some impurity in it. Experiment has not confirmed his statement. Zinc chlorid coagulates the blood and renders it much darker than usual. The highly vascular condition of the cinerea would soon render it susceptible to the action of this salt, and it would in general assume a shade relatively much darker than the alba. The sodium chlorid is supposed to render the zinc more soluble, and to some extent to lessen its causticity. The glycerin is also useful in this latter respect, but its chief use besides preservation is to bring the fluid up to the required specific gravity.

A one-fifth per cent. solution of picric acid in fifty per cent. alcohol has been used by Professor S. H. Gage with very successful results upon a human brain. The specimen was carried up gradually to 95% through the intermediate grades of alcohol. He has also obtained excellent preparations of fetal brains by injecting the preservative through a hypodermic needle into the brain cavities.

"Dry" preparations are those which may remain permanently exposed to the atmosphere at the ordinary temperature, without apparent detriment. There are essentially two methods of preparation, the one consisting of actual dessication or mummification, in which the specimens remain hard and

inflexible ; the other involves the infiltration of the tissue by some hygroscopic substance like glycerin which replaces the natural fluid by abstracting the requisite amount of moisture from the air. Such specimens, of course, are not dehydrated and therefore are not *dry* in the same sense as those of the former class.

A temporary dry preparation of the brain for demonstrative purposes has been recommended by von Lenhossek[22]. After thorough hardening in alcohol, the specimen, when needed for demonstration, is carefully dried in soft linen and then coated with a thin layer of celloidin applied with a fine brush. After five or ten minutes the celloidin dries, and as a thin, transparent, tough membrane affords great protection and firmness to the preparation. If exposed to the air for more than two hours the specimen will begin to shrink and should be returned to the alcohol.

Paraffin impregnation of brain tissue for dry preparations was first employed by Fredericq[7]. Schwalbe[18] in the same year (1876) adopted Fredericq's method slightly modified. The brain is hardened in zinc chlorid or alcohol, the membranes are removed and the specimen cut into suitable pieces, impregnation *in toto* does not seem to be advisable. After dehydrating in strong alcohol, immerse in turpentine until completely saturated, then infiltrate with soft paraffin at a temperature of 60° C. from five to eight days and let cool on a layer of cotton taking care to avoid deformation. W. C. Krauss[11] and others have employed a similar method and recommend it for friable specimens.

Dr. J. W. Blackburn's[1] method consists of allowing the specimen to harden for about five weeks in Müller's fluid, the pia being removed after a few days immersion. After thorough dehydration in alcohol it is placed in a saturated solution of Japan wax (a concrete oil, the product of *Rhus succedenea*) in chloroform. When the alcohol has been displaced the specimen is transferred to a bath of pure melted wax and kept there at the melting point (42° to 55° C.), until thoroughly infiltrated. Upon removal the wax drains from the surface leaving it perfectly smooth. A small proportion of paraffin will prevent cracking.

Stieda[19] immerses the brain in an aqueous solution of zinc chlorid for twenty-four hours, as soon as it becomes firm enough the pia is removed and the specimen is transferred to ninety-six per cent. alcohol for two or three weeks, to dehydrate, it is then transferred for an equal length of time to turpentine and finally immersed for two weeks or longer in the ordinary commercial oil-finish. It is laid on blotting paper to dry for about eight days, and acquires a dull brown color on its surface. A shrinkage occurs which he considers unimportant, about one fourth of the original volume being lost.

Teichman[19] has pursued a similar course, the difference being that the brains were hardened in alcohol and finally impregnated with "Damar-harz" or "Damar-lack."

So far as Stieda knows Broca was the first to use nitric acid for hardening the central nervous system. His formula is as follows:

Water.....5 parts.
Nitric acid.....1 part.

The brain is left in this mixture for two days; the quantity of the nitric acid is then doubled and after two days more the specimen is taken out and allowed to dry and harden. There is considerable shrinkage. A method of "galvanoplastie" devised by M. Oré[3] is said to give good and durable specimens. Duval has proposed a modification of Broca's method in that the specimen is finally to be infiltrated with paraffin.

Hyrtl[10] (1860) saw no special advantage in using salts or nitric acid combinations, and gave the preference to alcohol; the addition of sugar as recommended by Lobstein gives to the specimen a welcome degree of flexibility. His experiments on dry preparations were not wholly satisfactory; the brains of a horse and calf were utilized and after hardening in sublimate were "cooked" in linseed oil and then allowed to dry. They kept their shape for a couple of weeks but after some months the horse brain shrunk to the size of a small apple and that of the calf to the size of a nut.

Giacomini[9] was the first to use glycerin for "dry" preparations; his specimens have been highly commended for retain-

ing their volume and color to a remarkable degree. All glycerin methods are essentially the same in principle and differ from Giacomini's chiefly in the manner of hardening and manipulation. Giacomini prefers a saturated aqueous solution of zinc chlorid for hardening although potassium bichromate, nitric acid or alcohol will give good results. The pia is removed after an immersion of twenty-four hours in the zinc chlorid solution, the brain remains in the liquid for two or three days longer, until it tends toward the bottom of the vessel, when it should be removed, as a longer stay would cause it to absorb too much water, it is then transferred to 95 per cent. alcohol where it may remain indefinitely, ten or twelve days usually being sufficient. The specimen is finally put into pure glycerin or glycerin containing carbolic acid to the amount of one per cent., when it has sunk just below the surface it may be removed and exposed to the air. After a few days when the surface has become dry, it is varnished with india rubber or better yet with marine glue varnish diluted with a little alcohol. This completes the process.

Dissections should be made previous to the glycerin bath. Histological detail is also said to be preserved to a remarkable extent.

Laskowsky's[12] method consists of first washing the fresh specimen in water to remove the blood, it is then placed in the following mixture :

Water	100 parts.
95 % Alcohol	20 parts.
Boracic acid	5 parts.

Kept in a cool place.

The pia is removed and the brain then placed in a saturated alcoholic solution of zinc chlorid for five or six days, the bottom of the vessel being covered with cotton.

Transfer for fifteen or twenty days to a mixture consisting of :

Glycerin	100 parts.
Alcohol	20 parts.
Carbolic acid	5 parts.
Boracic acid	5 parts.

Let the specimen dry in the air, protected from dust.

Max Flesch[6] recommends the addition of one part of corrosive sublimate to three thousand parts of glycerin. A human brain he leaves in water for two days in order to wash out the blood, it is then placed in alcohol for four weeks; then for two weeks in equal parts of glycerin and alcohol and finally four weeks in pure glycerin, to every three thousand parts of which is added one part of corrosive sublimate (the sublimate is dissolved in a small quantity of water and alcohol and then added to the glycerin). Wherever it is necessary the brain is supported upon a layer of cotton to avoid deformity.

After the drainage of the superfluous glycerin the specimen is again placed for final storage upon a piece of blotting paper supported by a layer of cotton and the whole enclosed by a paste board box with a glass top, to protect from the dust. The expense is slight as the solutions can be used repeatedly. The alba and cinerea are said to remain well differentiated.

Struthers[20] hardens the brain in alcohol after the removal of the membranes, for ten or fourteen days. It is then put into :

Glycerin . . . . . 4 parts,
Carbolic Acid . . . . . 1 part.

for two or three days. When the superfluous glycerin drains off, the brain is put under a glass case in order that it may not take the dust. It is claimed that there is less shrinkage and more flexibility than in Giacomini's method.

Richardson[16] recommends the following formula :

Glycerin . . . . . 300 c. c.
Methylated spirit . . . . . 600 c. c.
Zinc chlorid . . . . . 2 grams.

" Dissolve the zinc chlorid in the spirit and gradually add the glycerin. In use immerse the structure in the solution and keep it in until it is fully saturated. Then remove and let harden," [dry].

As a result of numerous experiments and a careful study of previous methods, the following process was devised : The preliminary treatment is as directed on page 393. After dehy-

dration in repeated changes of ninety-five per cent. alcohol, immerse the brain in a mixture of:

Turpentine . . . . . 3 parts,
Castor oil . . . . . 1 part,

until it becomes tolerably translucent (one or two weeks) changing the solution if it becomes cloudy, then transfer to pure castor oil for a week or two. Allow it to drain on a layer of cotton covered with absorbent paper until the surface dries and then paint it over a few times with an alcoholic solution of bleached shellac. The specimen soon becomes firm and requires no special attention when once it has become dry. This process differentiates alba and cinerea well. (See Plate). The brain sections or dissections should be made before immersing in the turpentine-oil mixture. It will be found that the alba becomes translucent first, the preparation at this particular stage may then be put into the pure castor oil until thoroughly penetrated and subsequently drained and shellaced. The castor oil may be used repeatedly and costs only one-half as much as glycerin.

Some shrinkage occurs, the dry specimen losing about one-fourth of its volume after it has left the liquid. It should be remembered that the brain consists of eighty-eight per cent of fluid and that the possibilities of evaporation and the replacement of this natural liquid by an artificial one as in dehydration render some shrinkage inevitable. It is not feasible therefore to harden a brain rapidly without some condensation of tissue, the main point is to harden the specimen without distortion or to have the shrinkage evenly distributed. Theoretically the shrinkage might be lessened or entirely obviated if each fluid or mixture into which the brain is immersed could be kept at the same specific gravity as the brain itself, and replace equally its normal fluid. This does not seem to be practicable where dehydration is necessary. The dry process has given good results on delicate fetal brains, it seems to strengthen them so that they may be readily handled, but great care must be taken in transferring them through the different fluids. If breakage should occur the

parts may be stuck together with mucilage and after shellacing again the specimen will be as durable as ever.

There are objections to both the dry and glycerin methods. The former renders the specimens too hard and there is perhaps a little more shinkage; with the latter there is more flexibility but there is a greasy and disagreeable feel to the preparations. Experiments are in progress with a view toward combining the more desirable features of each, by compounding an emulsion in the following proportions:

Glycerin . . . . . 100 c. c.
Castor oil . . . . . 100 c. c.
Gum arabic . . . . . 50 grams
or,
Gum tragacanth . . . . . 50 grams

If well made it does not "crack" and seems to penetrate the tissues quite well though somewhat slowly. The emulsion can be used repeatedly by rubbing it up again in a mortar before putting a new specimen into it. The brain may be shellaced as in the previous method.

The writer wishes to acknowledge his obligations to Professor Wilder whose kindly interest in this line of work has rendered practicable many interesting experiments and whose indefatigable energy in scientific research has been an example as well as an incentive in the preparation of this paper. Acknowledgments are also due to Professor H. H. Donaldson of Chicago University and to Professor S. H. Gage of Cornell for valuable suggestions.

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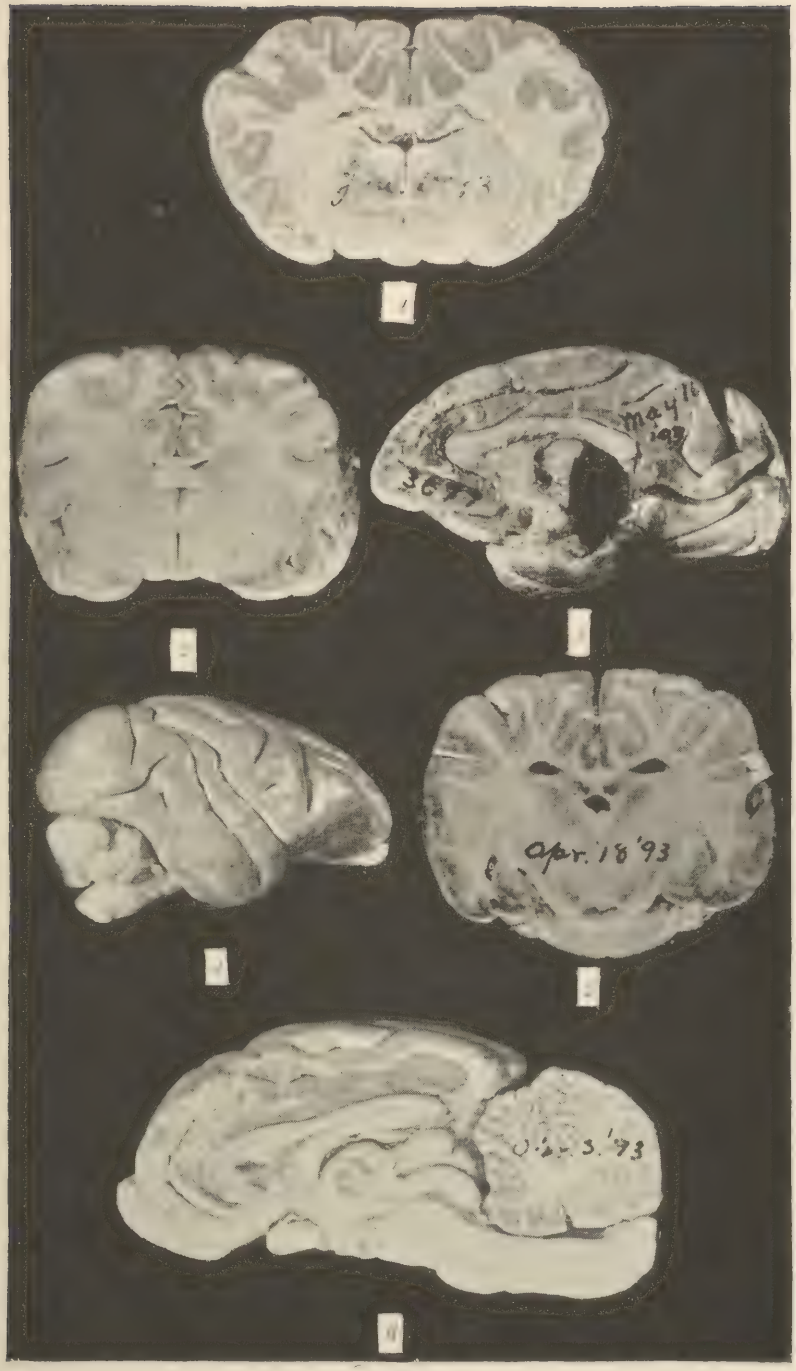
DESCRIPTION OF PLATE.

All of the figures are at about the natural size of the dry specimens and were prepared according to the castor oil method.

The transections show the differentiation of the alba and cinerea.

Fig. 1 and 6 are from the sheep, *Ovis aries*. Fig. 2 and 5 are from the dog, *Canis familiaris*. Fig. 3 is the mesal view of the right hemicerebrum of *Macacus rhesus*. Fig. 4 is the lateral aspect of the right hemicerebrum of *Macacus cynomolgus*.



DRY PREPARATIONS OF THE BRAIN—FISH.

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